A Fatal Case of Pulmonary Penicilliosis

Takeshi Mori¹⁾, Makiko Matsumura¹⁾, Tomoo Kohara¹⁾, Yoshirou Watanabe¹⁾,
Taijiro Ishiyama¹⁾, Yoshihisa Wakabayashi¹⁾, Hideo Ikemoto¹⁾,
Akiko Watanabe²⁾, Masataka Tanno³⁾, Toshikazu Shirai³⁾
and Masakatsu Ichino⁴⁾

Department of Internal Medicine¹⁾, Central Laboratory for Medical Sciences, Division of Pathobiology²⁾ and
Department of Pathology³⁾, Juntendo University School of Medicine, 2-1-1 Hongo, Bunkyo-ku, Tokyo 113, Japan
Division of Microbiology⁴⁾, National Institute of Hygienic Sciences, 1-18 Kamiyoga, Setagaya-ku, Tokyo 158 Japan

[Received for Publication: September 3, 1987]

A 19-year-old male developed acute lymphocytic leukemia complicated with pulmonary penicilliosis. Marked leukopenia with a high fever continued for more than two weeks. A strain of *Candida glabrata* was isolated from blood culture. Treatment was initiated by combination therapy with antifungal drugs and anticancer drugs. To treat the suspected infection with this fungus, oral flucytosine and intravenous miconazole were administered, but the clinical symptoms were not alleviated. Thus oral fluconazole was added to the regimen. X-ray examinations revealed that the infiltration in the middle and lower fields of the right lung gradually became localized, and the findings suggested the formation of a fungus ball. The patient died of right pneumothorax on the 66th hospital day.

At autopsy, mass lesions were detected in the thoracic cavity about $4 \times 4 \times 5$ cm in size in the right middle lobe and about $1 \times 1 \times 3$ cm in size in the left upper lobe. These mass lesions were filled with necrotic lung tissues, and they thus differed in nature from the so-called fungus ball. Those lesions and the cavity wall contained numerous fungal elements consisting of dichotomous septated hyphae. Two morphologically different fungi were isolated from the mass lesion in the right lung: one isolate was identified as *Penicillium citrinum* and the other was a *Penicillium* species without reaching the final identification. The morphology of these two isolates was examined in detail with an electron microscope.

This seems to be the first reported case of pulmonary penicilliosis due to *P. citrinum* and an unidentified *Penicillium* sp.

Key words: Pulmonary penicilliosis, *Penicillium citrinum*, scanning electron microscopy

Penicillium is usually a saprophytic and non-pathogenic organism which is widely present in the environment and often isolated from human sources. On the other hand, it is known to be a causative organism of infections of the ear, sinus, eye^{1,2)} and urinary tract³⁾, endocarditis of aortic valve prosthesis⁴⁾, pulmonary infections and allergic bronchopulmonary disease⁵⁾, but it very rarely causes systemic mycoses.

In this report, we describe a case of acute lymphocytic leukemia complicated with fatal penicilliosis. This is thought to be the first report of a pulmonary infection due to two different strains of *Penicillium*: *P. citrinum* and an unidentified *Penicillium* sp.

Case Report

A 19-year-old male:

The patient was well prior to November of 1984, when he complained of a fever and lumbago. A local hospital suspected acute leukemia, and the patient was admitted to our hospital on December 15, 1984. Based on the results of bone marrow puncture, he was diagnosed as having acute lymphocytic leukemia and was treated with combination chemotherapy consisting of vincristine (V), prednisolone (P) and aclacinomycin (A). Following the chemotherapy, he achieved complete remission and was discharged on February 5, 1985. Due to a relapse, he was readmitted from September 17 through November 2, 1985. He responded well to the VPA combination chemotherapy and a high dose of 1-aspar-

* To whom inquiries should be addressed.

aginase, again achieving complete remission. The third admission due to relapse was from February 10 to May 27, 1986. Treatment with vindesine, adriamycin and prednisolone, and a high dose of cytosine arabinoside achieved partial remission. The patient was discharged at his own request. However, due to repeated fever and back pain, he was readmitted on June 4, 1986. Laboratory examinations showed RBC $342 \times 10^4/\text{mm}^3$, Hb 10.2 g/dl, Ht 31.1%, WBC $6400/\text{mm}^3$ (Band 10.5, Seg 62, Lymph 12.5, Mono 4, Eosino 0.5, Baso 0.5, myelocyte 0.9 and myeloblast 9.5%) and platelets $16.6 \times 10^4/\text{mm}^3$. As a result of combination therapy with enocitabine, daunorubicin, vindesine, 6-mercaptopurine and prednisolone, the leukocyte count decreased to under $400/\text{mm}^3$, but the myeloblast count increased to 32%. Four days later, the leukocyte count became $100/\text{mm}^3$, and this condition continued for 18 days and recovery of the bone marrow function was delayed. During this condition, a high fever of 38°C to 39°C persisted. From the time of admission, amphotericin B syrup (2400 mg/day) had been administered, but the fever did not abate and blood culture was positive for *Candida glabrata*. Chest X-ray films showed infiltration in the middle and lower fields of the right lung. These findings suggested fungal pneumonia, and therapy with miconazole (1200 mg/day, i.v.) and flucytosine (400 mg/day, p.o.) was started. In spite of this combination therapy, the clinical symptoms did not subside.

Then oral fluconazole (100 mg/day) was added 4 days later. Three hours after the final dosing of fluconazole administered for 10 consecutive days, the serum concentration reached a relatively high level of $7.0 \mu\text{g/ml}$ measured by high-performance liquid chromatography.

The shadows in the middle and lower lung fields of the right lung gradually became localized, and their borders became apparent (Fig. 1). On July 10, a complete blood count showed a tendency for WBC to rise, as did myeloblasts. On July 24, chest X-rays showed a crescent-shaped radiolucency surrounding the pre-existing parenchymal densities, i.e., the so-called meniscus sign. On July 28, a mass lesion was recognized in the cystic shadow, which gradually became oval and extended (Fig. 2), but the mass lesion tended to decrease in size (Fig. 3). At the same time, a small area of infiltration was seen in the left upper lung field. On August 8, the patient

Fig. 2. Chest X-ray film taken on July 28, 1986.

Fig. 1. Chest X-ray film taken on July 9, 1986.

Fig. 3. Chest X-ray film taken on August 6, 1986.

suddenly complained of right colicky chest pain and dyspnea. Chest X-ray films showed right pneumothorax and deviation of the mediastinal shadow to the left. The patient's condition deteriorated, and he died due to acute respiratory failure on August 9, 1986.

Autopsy Findings

The bone marrow was found to be filled with leukemic cells, leading to a diagnosis of acute lymphocytic leukemia.

The right lung had contracted to 11×4 cm in size. There were mass lesions in the cavities; they measured as large as $4 \times 4 \times 5$ cm in the right middle lobe and $1 \times 1 \times 3$ cm in the left upper lobe. The cause of death was considered to be rupture of the cavity wall surrounding the mass lesion in the right middle lobe (Fig. 4).

Two strains of *Penicillium* were isolated from the lesion in the right lung at autopsy.

In the cavity wall, a thrombosed blood vessel was seen which contained many fungal elements (Fig. 5). The mass lesions showed necrosis of the lung tissue which exposed the alveolar structure where the fungal elements penetrated the blood vessels. Thus, these lesions were considered to be clearly different from a fungus ball. The fungal elements were also seen in the wall of

Fig. 5. Macroscopic view of the cavity wall (PAS staining).

Fig. 4. Gross appearance of the right lung. Cavity wall and intracavitary mass are seen.

Fig. 6. Methenamine-silver stain of the mass lesion. (original magnification $\times 200$).

the ectatic bronchi connected to the cavity, and they penetrated and obstructed the blood vessels in the cavity wall. In the lung tissue surrounding the cavity, leukemic cells infiltrated the alveolar septa.

A state of cytomegalic inclusion disease was observed.

The fungal elements were recognized as dichotomous branching septated hyphae (Fig. 6). Scanning electron microscopy revealed that the fungal elements in the mass lesions developed in all directions in the remaining alveolar structure. There were spaces between the

Fig. 7 Scanning electron microscopy of a fungal element developing in the space between the cavity wall and the mass lesion.

cavity wall and the mass lesions, and the fungal elements developed into the space from both sides. They were spore-bearing hyphae and had conidia occurring in unbranched chains cut off from the tip of sterigmata which were verticillately arranged from the ends of metulae (Fig. 7). These findings suggested that the fungi in the lesions were species of *Penicillium*, and not species of *Aspergillus* since no vesicles were recognized.

Mycology of the Isolates

When a small number of conidia of the isolate considered to be *Penicillium citrinum* was inoculated onto the center of a Czapek-Dox agar plate and incubated at 25°C for 2 weeks, a round, flat colony 55 mm in diameter developed (Fig. 8). The upper side of the colony had a blue-green color and a velvet-like appearance, of which the bottom side was yellowish-brown. The conidiophores were long (23 μ m), and their surface was smooth. From the end of each conidiophore, 3 to 5 vertically-arranged metulae arose to form the phialide. The conidia were oval, spherical or semi-spherical, and their surface was smooth. The periphery of the colony and its reverse were luminated and pigmented in citrinum color. This fungus grew well at 37°C. Based on these features, this isolate was identified as *Penicillium citrinum* (Fig. 9).

The colony of the other isolate grown under the same culture conditions as described above was round and

Fig. 8. Colony of *P. citrinum* on Czapek-dox agar after inoculation at 25°C for 2 weeks.

Fig. 9. Light microscopic features of *P. citrinum* cultured from the right middle lobe of the patient. (original magnification $\times 200$)

flat, 70 mm in diameter (Fig. 10); the obverse showed a brownish-green surface, a white periphery and a light-brown reverse. The conidiospores were long (22 μ m), branched and had smooth surfaces. The penicillus was monoverticillated. The conidia were spherical with smooth surfaces. This isolate did not grow on Czapek-Dox agar at 37°C. These features indicated that the fungus belongs to the genus *Penicillium*, but no final identification was made (Fig. 11).

The minimal inhibitory concentrations (MICs) of amphotericin B, miconazole and flucytosine for the isolates were tested by the agar dilution technique⁶⁾. The MICs of amphotericin B, miconazole and flucytosine against *P. citrinum* were 12.5, 0.78 and over 800 μ g/ml, while the MICs against the unidentified *Penicillium* were 0.78, 0.39 and 0.78 μ g/ml. However, the twenty-five percent inhibitory dose of fluconazole against the *Penicillium* spp. was over 80 μ g/ml measured by the micro-liquid

Fig. 10. Colony of unidentified *Penicillium* sp. after inoculation at 25°C for 2 weeks.

Fig. 11. Light microscopic features of unidentified *Penicillium* sp. cultured from the right middle lobe of the patient. (original magnification $\times 200$)

dilution method with an auto-reading system⁷⁾.

Discussion

In this case, the morphology of the fungi in the pulmonary lesions and on mash preparations closely resembled the *Penicillium* species detected at autopsy. Therefore, it was surmised that this case had been infected by *Penicillium*. *Candida glabrata* was detected once and disappeared after the antifungal therapy; it might have been a contaminant.

Okudaira *et al.*⁸⁾ reported that *Aspergillus* and *Penicillium* were most frequently isolated from normally aerated lungs in cases of sudden and unexpected death or accidental death without any pathological findings in the lung. However, it is well known that the respiratory parenchyma of healthy humans is not aseptic, and that *Penicillium* and *Aspergillus* actually inhabit the normal respiratory tract. It may be justifiable to assume that

these fungi can produce opportunistic infections in these organs and tissues, particularly in immunodeficient patients.

The first case of bronchopulmonary penicilliosis was reported by Castellani⁹⁾ in 1918, and since then at least 20 cases of such infection with mycological evidence have been described in the literature (Table 1). Eight of those cases occurred before the advent of antibiotics, and the 12 cases without underlying disease or with pulmonary aspergilloma were associated with a fungus. It should be noted that all the cases with underlying diseases such as leukemia, lymphoma or systemic lupus erythematosus described in those papers ended in death.

P. crustaceum and *P. marneffei* have been reported to be the most common species isolated from human sources. Our present case seemed to be the first one with pulmonary lesions caused by two species of the genus *Penicillium*, i.e., *P. citrinum* and another *Penicillium* sp., and concomitant cytomegalic inclusion disease.

Gilliam and Vest³⁾ reported a case of *P. citrinum* infection of the urinary tract in which the organism was isolated from the kidney and urinary bladder.

Since the introduction of antibiotics, antimetabolites and steroids, secondary mycotic infections in leukemias, lymphomas, and cancers have been increasing in number. This has been attributed to lowering of the body resistance due to these underlying diseases, particularly when there is also the undesirable influence of therapy with antimetabolites, steroids or both. Changes in the balance of the normal microbial flora due to antibiotic therapy have also been regarded as one of the most important factors leading to such opportunistic infections.

In our case also, generalized debilitation, severe leukopenia and steroid therapy might be implicated as factors that predisposed the patient to *Penicillium* infection and aggravated the severity of the disease, including the infection.

Huang and Harris¹⁰⁾ reported a case of pulmonary and cerebral infection by *P. commune* with concomitant gastrointestinal candidiasis. In penicilliosis, which resembles aspergillosis, the causative agent has been known to possess the ability to invade the blood vessels, resulting in thrombosis, hemorrhage and infarction. All of these pathological features are indistinguishable from those caused by *Aspergillus*.

Curtis *et al.*¹¹⁾ described that in the vasoinvasive form of aspergillosis, thrombosis of blood vessels and infarction of the central portion of the inflammatory focus occur. With retraction of the infarcted center or resorption of necrotic tissue at the periphery, a sequestrum of devitalized lung tissue infiltrated with fungi is formed. Subsequent filling lucency by air of the space between

Table 1. Reported Cases of Bronchopulmonary Penicilliosis

No.	Author	Year	Age	Sex	Species of <i>Penicillium</i>	Predisposing factor	Outcome
1	Castellani ⁽⁹⁾	1918		M	<i>P. crustaceum</i>	No underlying disease	Recovered with KI therapy
2	Giodano ⁽¹⁴⁾	1918		M	<i>P. crustaceum</i>	No underlying disease	Recovered with KI therapy
3	Pezzali ⁽¹⁵⁾	1921		M	<i>P. crustaceum</i>	Chronic Bronchitis	Died of ruptured syphilitic aortic aneurysm
4	Nußbaum <i>et al</i> ⁽¹⁶⁾	1927	58	M	<i>P. bicolor</i> (Fries)	Inhalation of wood dust	Died without therapy
5	Talice <i>et al</i> ⁽¹⁷⁾	1929	58	M	<i>P. bertai</i>	No underlying disease	Improved by KI therapy
6	Nino ⁽¹⁸⁾	1932	42	M	<i>P. crustaceum</i>	No underlying disease	Improved by KI therapy
7	Aimé <i>et al</i> ⁽¹⁹⁾	1933	41	F	<i>P. crustaceum</i>	No underlying disease	Improved by KI therapy
8	Da Silva ⁽²⁰⁾	1937	23	M	<i>P. glaucum</i>	Inhalation of corn dust	Recovered with KI therapy
9	Garretón ⁽²¹⁾	1945	52	M	<i>Penicillium</i> species not identified	Chronic bronchitis	Improved by KI therapy
10	Delore <i>et al</i> ⁽²²⁾	1955	46	M	<i>P. spinulosum</i>	Chronic bronchitis	Improved by combined KI and cortisone therapy
11	Huang <i>et al</i> ⁽¹⁰⁾	1962	42	M	<i>P. commune</i>	Acute leukemia with steroid and antibiotic therapy	Died of mycotic and bacterial infections
12	Ikemoto <i>et al</i> ⁽²³⁾	1967	79	M	<i>P. nigricans</i> (<i>A. candidus</i>)	Pulmonary aspergilloma	No therapy
13	Sahn <i>et al</i> ⁽⁵⁾	1973	76	M	<i>P. rubrum</i>	Allergic bronchopulmonary penicilliosis	Bronchial hygiene consisting of inhalation of nebulized isoetharine and water
14	Liebler <i>et al</i> ⁽²⁴⁾	1977	34	M	<i>Penicillium</i> species not identified	No underlying disease	Wedge resection of <i>Penicillium</i> granuloma in the posterior segment of the right upper lobe
15	Joyanetra <i>et al</i> ⁽²⁵⁾	1984	6	F	<i>P. marneffeii</i>	No underlying disease	Cured with AMPH therapy
16			35	M	<i>P. marneffeii</i>	Tuberculosis	Died without therapy
17			50	F	<i>P. marneffeii</i>	No underlying disease	Improved by combined AMPH and 5-FC therapy
18			38	M	<i>P. marneffeii</i>	Lymphoproliferative disorder	Died by combined AMPH and 5-FC therapy
19			25	F	<i>P. marneffeii</i>	Systemic lupus erythematosus and pregnancy	Died without therapy
20	Tanphaichitra <i>et al</i> ⁽²⁶⁾	1984	60	F	<i>P. marneffeii</i>	Abscess with granulomatous inflammation; osteomyelitis & salmonellosis	Improved by combined AMPH and 5-FC therapy
21	This article	1987	19	M	<i>P. citrinum</i> and unidentified <i>Penicillium</i> sp	Acute lymphocytic leukemia	Died in spite of therapy with AMPH (p.o.) and imidazole derivatives.

KI: potassium iodide, AMPH: amphotericin B, 5-FC: flucytosine, p.o.: oral route

the sequestrum and peripheral lung tissue results in an air crescent, as has been observed radiographically.

In the case of functional disorders of cellular immunity and macrophages, especially in leukemia, the ability to phagocytize fungi and suppress the growth of fungi is reduced. The fungi in such cases may remain viable even when their activity is decreased by antifungal drugs.

It is well known that penicillin is obtained from *P. notatum*, and some *Penicillium* sp. produce mycotoxin. Mycotoxin might influence the host by suppressing immunity. *P. citrinum* produces citrinin, a kind of mycotoxin, which has nephrotoxicity. But in this case, renal damage was not recognized.

The activities of several antifungal drugs against *P. citrinum* and the unidentified *Penicillium* sp. were tested. To date, it has been said that amphotericin B is hardly absorbed by the oral route. In recent studies¹²⁾, however, oral administration of high-dose amphotericin B resulted in an elevated serum drug level. Nevertheless, in our case, oral amphotericin B was ineffective against the infection with the two *Penicillium* species.

Because flucytosine and fluconazole also showed little or no activity against our isolates, these antifungals could not be expected to achieve a good effect in clinical application. On the other hand, in view of a report¹³⁾ that repeated drip infusion of miconazole provided a significant serum concentration, we thought this might be efficacious in our case. It had, however, no effect.

It has already been reported that amphotericin B, miconazole and fluconazole, which we administered to our patient, are all antifungals that inhibit fungi by suppressing sterol synthesis in the membranes and that the actions of these drugs are competitive with each other. The failure of these drugs to cure our case infected with *Penicillium* spp. might derive from their mechanism of action.

We gratefully acknowledge the kind guidance and invaluable advice provided by Dr. Kazuo Iwata, M.D., professor emeritus of Tokyo University.

References

- 1) Gugnani, H.C., Talwer, R.S., Njoku-Obi, A.N.U. and Kodilinye, H.C.: Mycotic keratitis in Nigeria; a study of 21 cases. *Brit. J. Ophthalmol.*, **6**: 607—613, 1976.
- 2) Eschete, M.L., King, J.W., West, B.C. and Oberle, A.: *Penicillium chrysogenum* endophthalmitis. *Mycopathologia*, **74**: 125—127, 1981.
- 3) Gilliam, J.S. Jr. and Vest, S.A.: *Penicillium* infection of the urinary tract. *J. Urol.*, **65**: 484—489, 1951.
- 4) Upshaw, C.B. Jr.: *Penicillium* endocarditis of aortic valve prosthesis. *J. Thorac. Cardiovasc. Surg.*, **68**: 428—431, 1987.
- 5) Sahn, S.A. and Lakshminarayan, S.: Allergic bronchopulmonary penicilliosis. *Chest*, **63**: 286—288, 1973.
- 6) Hiratani, T. and Yamaguchi, H.: Laboratory assessment of a systemic antimycotic miconazole (Base); *in vitro* activity. *Chemotherapy*, **32**: 534—540, 1984.
- 7) Yamaguchi, H.: in preparation.
- 8) Okudaira, M., Kurata, H. and Sakabe, F.: Studies on the fungal flora in the lung of human necropsy cases; a critical survey in connection with the pathogenesis of opportunistic fungal infections. *Mycopathologia*, **61**: 3—18, 1977.
- 9) Castellani, A.: The higher fungi in relation to human pathology. *Lancet*, **1**: 895—901, 1920.
- 10) Huang, S. and Harris, L.S.: Acute disseminated penicilliosis; report of a case and review of pertinent literature. *Amer. J. Clin. Path.*, **39**: 167—174, 1963.
- 11) Curtis, A.M., Walker-Smith, G.J. and Ravin, C.E.: Air crescent sign of invasive aspergillosis. *Radiology*, **133**: 17—21, 1979.
- 12) Shibuya, T., Morioka, E., Ohhara, N. and Niho, Y.: Clinical study for prevention of deep fungal infection by orally administered large dose of amphotericin B. *J. Jpn. Assoc. Infect. Dis.*, **61**: 287—291, 1987.
- 13) Uchida, K. and Yamaguchi, H.: Bioassay for miconazole and its level in human body fluids. *Chemotherapy*, **32**: 541—546, 1984.
- 14) Giodano, M.: Un caso di micosi polmonare da "*Penicillium alaucum*" *Ann. med. nav. Roma*, **2**: 912—918, 1918.
- 15) Pezzali, G.: Contributo di casistica clinica; un caso di penicilloso del polmone secondaria. *Clin. vet.*, **44**: 201—205, 1921.
- 16) Naßbaum, R. and Benedek, T.: Pneumonomycosis penicillina, eine Gewerbekrankheit zum Kapitel der Lungengeschwulste. *Beitr. Klin. Tuberk.*, **67**: 756—770, 1927.
- 17) Talice, R.V. and Mackinnon, J.E.: *Penicillium bertai* (I), n. sp. agent d'une mycose broncho-pulmonaire de l'homme. *Ann. parasitol.*, **7**: 97—106, 1929.
- 18) Nino, F.L.: Broncomycosis penicilliar. *Semana. méd.*, **2**: 1015—1020, 1932.
- 19) Aimé, P., Greuzé, P. and Kresser, H.: Mycose pulmonaire à "*Penicillium crustaceum*" avec signes cliniques et aspect radiologique d'abcès du poumon. *Presse méd.*, **41**: 761—763, 1933.
- 20) Da Silva Lacaz, C.: Considerações sobre um caso di penicilliose pulmonar. *Hospital, Rio de Janeiro.*, **15**: 327—339, 1939.

- 21) Garretón, U.I.: Un caso de bronquitis micotica a *Penicillium*. An. Med. Concepcion, **2**: 103—105, 1945.
 - 22) Delore, P., Coudert, J., Lambert, R. and Fayolle, J.: Un cas de mycose bronchique avec localisations musculaires septicémiques. Presse méd., **63**: 1580—1582, 1955.
 - 23) Ikemoto, H., Watanabe, K. and Hisauchi, O.: Intracavitary fungus ball; report of three new cases, including one in which both *Aspergillus candidus* and *Penicillium nigricans* were obtained by sputum culture. Jpn. J. Chest. Dis., **26**: 291—296, 1967.
 - 24) Lieber, G.A., Magovern, G.J., Sidighi, P., Park, S.B. and Cushing, W.J.: *Penicillium* granuloma of the lung; presenting as a solitary pulmonary nodule. JAMA., **237**: 671, 1977.
 - 25) Joyanetra, P., Nitiyanant, P., Ajello, L., Padhye, A.A., Lolekha, S., Atichartakarn, V., Vathesatogit, P., Sathaphatayavongs, B. and Prajaktam, R.: Penicilliosis marneffeii in Thailand; report of five human cases. Amer. J. Trop. Med. Hyg., **33**: 637—644, 1984.
 - 26) Tanphaichitra, D. and Srimuang, S.: Cellular immunity (T-cell subset using monoclonal antibody) in tuberculosis, melioidosis, pasteurellosis, penicilliosis; and role of levamisole and isoprinosine. Develop. Biol. Standard, **57**: 117—123, 1984.
-